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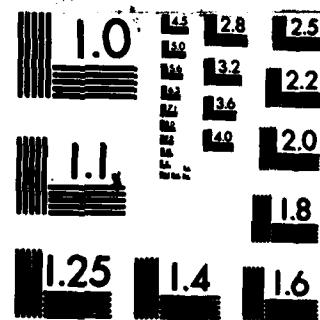
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A SIX-MONTH CLINICAL EVALUATION OF DECALCIFIED FREEZE-DRIED BONE  
ALLOGRAFTS IN PERIODONTAL OSSEOUS DEFECTS

by

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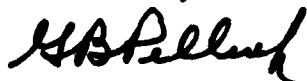
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Submitted by:



G. B. Pelleu, Jr., Ph.D.  
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# A Six-Month Clinical Evaluation of Decalcified Freeze-Dried Bone Allografts in Periodontal Osseous Defects\*

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THE OSTEOGENIC POTENTIAL of decalcified freeze-dried bone allografts in the treatment of human periodontal osseous defects was evaluated over a 6-month period. Cortical bone, obtained under sterile conditions from a human donor within 24 hours after death, was decalcified, freeze-dried and ground to a particle size of 250 to 500  $\mu\text{m}$ . Twenty-seven osseous defects with one-, two- and wide three-wall morphology were treated. Clinical measurements were made with a stent and a calibrated periodontal probe before surgery, at the time of surgery, and at re-entry. The combined mean osseous regeneration for all defects was 2.4 mm. This represented a 65% mean bone-fill of the original defect. The findings demonstrate that decalcified freeze-dried bone allograft has potential as an osseous grafting material in periodontal therapy.

Autogenous bone has been used with clinical success in the treatment of periodontal osseous defects for many years.<sup>1-3</sup> However, there are certain limitations. Procuring it often necessitates an additional surgical procedure that may result in increased postoperative morbidity, and there may be insufficient quantities of autogenous bone for grafting large or multiple defects. As an alternative, bone allografts have been utilized. One type of allograft is decalcified freeze-dried bone.

Urist and co-workers,<sup>4-6</sup> using cortical bone which was decalcified with hydrochloric acid and then freeze-dried, have reported the induction of new bone formation in various heterotopic sites in animals. In orthotopic sites, decalcified bone allografts have consistently resulted in

complete bridging of ulnar gap defects<sup>10</sup> and in complete filling of experimental parietal wounds with new bone.<sup>11</sup> Decalcified allogeneic bone has compared favorably with autogenous bone when used in experimental mandibular defects in dogs<sup>12-15</sup> and monkeys.<sup>16</sup> In addition, decalcified freeze-dried bone allografts have been used successfully to reconstruct defects of the maxilla and mandible in humans.<sup>17, 18</sup>

Libin et al.<sup>19</sup> evaluated decalcified freeze-dried bone allografts for use in treatment of three osseous defects in three patients. New bone was formed and there was a gain in attachment in all grafted areas. More recently it has been reported that significant gains in clinical attachment were achieved in periodontal osseous defects grafted with decalcified freeze-dried bone allografts but not in comparable defects treated by flap and curettage only.<sup>20</sup> The purpose of the present study was to evaluate clinically the osteogenic potential of decalcified freeze-dried bone allografts in the treatment of human periodontal intrasosseous defects.

## MATERIALS AND METHODS

Cortical bone was obtained under sterile conditions from the femur of a human donor within 24 hours after death. It was defatted in chloroform-methanol for 6 hours at 25°C, autoclaved in phosphate buffer containing iodoacetic acid and sodium azide for 72 hours at 37°C, and demineralized in 0.6 N hydrochloric acid for 72 hours at 4°C after a modification of the technique developed by Urist and co-workers.<sup>21</sup> The decalcified tissue was then frozen at -197°C for 4 weeks and freeze-

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Figure 1. Stent with calibrated periodontal probe in place measuring loss of attachment from a fixed reference point (base of stent).

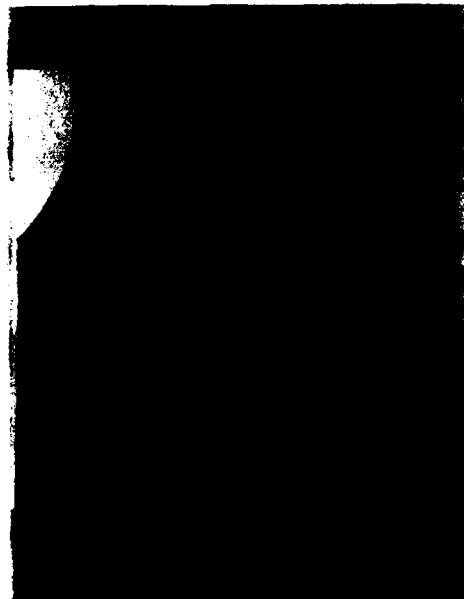


Figure 2. Measure of attachment loss: (1) base of stent to cementoenamel junction, (2) base of stent to free gingival margin, (3) base of stent to base of pocket.

dried according to the protocol of the Navy Tissue Bank.<sup>22</sup> The allogeneic bone was ground under sterile conditions in a bone mill\* and sieved to a particle size ranging from 250 to 500  $\mu\text{m}$ . The graft material was placed in  $\frac{1}{2}$ -oz sterile glass bottles, and samples were cultured, subjected to a secondary vacuum, sealed and stored at room temperature.

Five periodontists evaluated the material, following the designated protocol of this study. Each was required to document the management and regenerative response



Figure 3. Osseous measurements: (1) base of stent to alveolar crest, (2) base of stent to base of bone defect.

Table 1  
*Osseous Regeneration in Intraseptal Defects Treated with Decalcified Freeze-Dried Bone Allografts\**

Type of defect	No. of defects	Initial depth (mm)		Osseous regeneration (mm)		Bone fill (%)
		Mean	Range	Mean	Range	
One-wall	5	4.4	2.0-8.0	2.6	1.0-6.0	61
Two-wall	14	3.0	1.0-9.0	1.8	1.0-4.0	62
Three-wall	8	4.0	2.0-7.0	2.9	1.0-6.0	73
Total	27	3.8	1.0-9.0†	2.4	1.0-6.0†	65

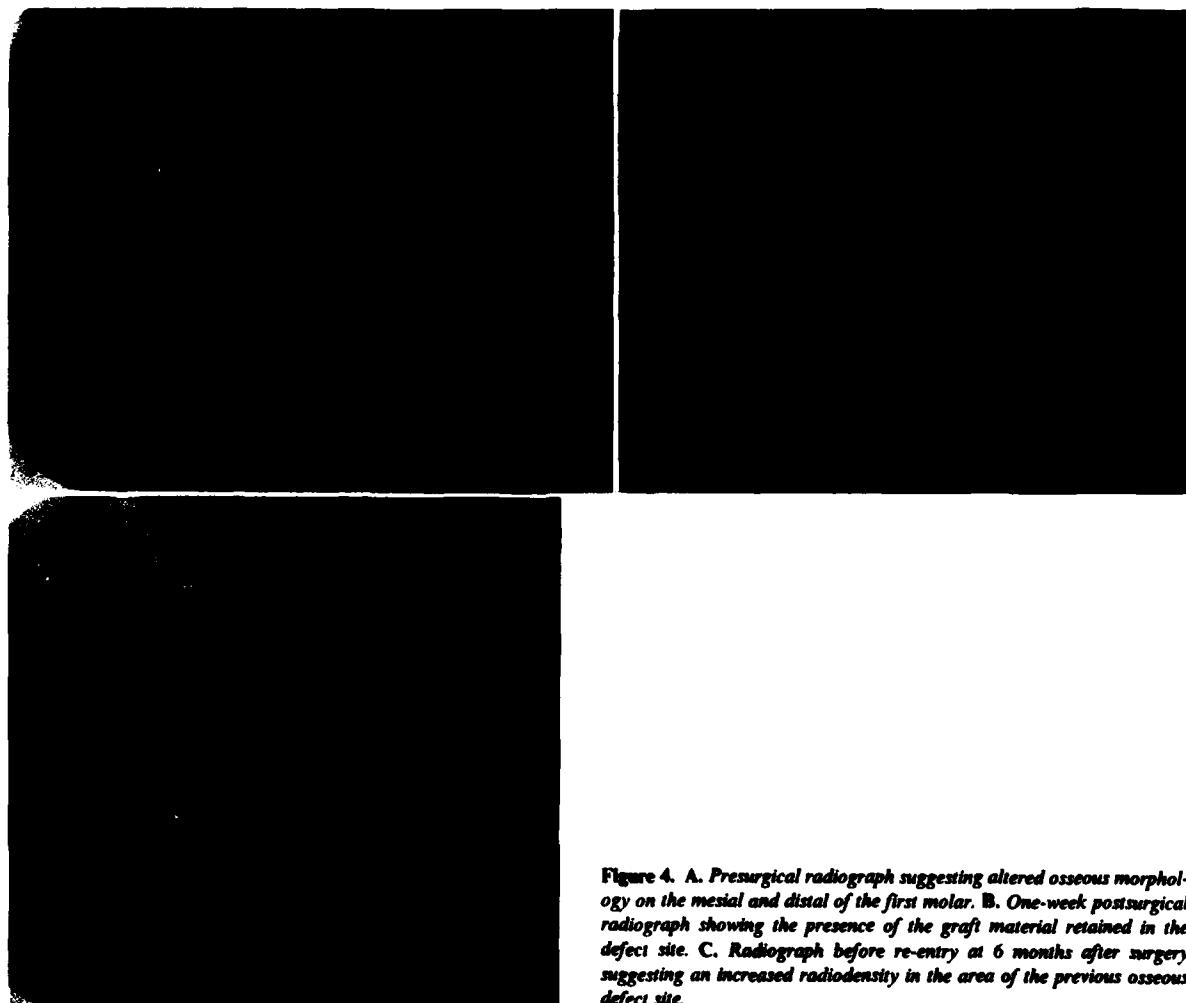
\* Eleven subjects.

† Combined mean and range for the three types of defects.

of his case. Presurgical management included patient demonstration of effective plaque control, scaling, root planing, prophylaxis and testing the vitality of involved teeth. Each clinician was given the option of occlusal adjustment, antibiotic coverage, flap design and recall regimen as dictated by treatment requirements.

Decalcified freeze-dried bone allografts were used in one-, two- and wide three-wall defects as described by Goldman and Cohen.<sup>23</sup> Each clinician was required to record measurements made before surgery, at the time of surgery, and at reentry to document the osseous changes effected by a prescribed methodology. The amount of regeneration was measured. A stent was used along with a calibrated periodontal probe to ensure reliability and reproducibility of data collected sequentially (Fig. 1). Presurgical measurements were made from the base of the stent to the cementoenamel junction, to the free gingival margin and to the base of the pocket (Fig. 2). Osseous measurements were made from the base of the stent to the alveolar crest and from the base of the stent to the base of the intraseptal defect (Fig. 3). Each defect was re-entered 4 to 6 months after the grafting, and all preceding measurements were repeated. Clinical data were supplemented with radiographs and photographs.

\* Tekmar Model A-20, Tekmar Co., Cincinnati, OH.



**Figure 4.** A. Presurgical radiograph suggesting altered osseous morphology on the mesial and distal of the first molar. B. One-week postsurgical radiograph showing the presence of the graft material retained in the defect site. C. Radiograph before re-entry at 6 months after surgery suggesting an increased radiodensity in the area of the previous osseous defect site.

## RESULTS

The measurements of the amounts of osseous regeneration obtained in intraosseous defects treated with decalcified freeze-dried bone allograft are tabulated in Table 1. Twenty-seven defects in 11 patients were grafted. There was a mean regeneration of 2.6 mm in one-wall defects, 1.8 mm in two-wall defects, and 2.9 mm in wide three-wall defects. This represented a mean fill of the defect of 61%, 62% and 73%, respectively. The overall mean for the 27 defects was 2.4 mm of osseous regeneration, or a 65% fill of the defect. Crestal apposition of new bone was noted in one two-wall and two three-wall defects. Each of these sites demonstrated 1.0 mm of new bone coronal to the original osseous crest. Radiographic and photographic documentation also suggested that a significant amount of osseous regeneration had taken place (Figs. 4 and 5).

Documentation of clinical soft-tissue attachment is presented in Table 2. The overall mean increase in clinical soft-tissue attachment was 1.9 mm, with a range

of -1.0 to 7.0 mm. Loss of attachment was noted in only two of the 27 treated defects.

## DISCUSSION

The results of this 6-month study confirm other reports indicating that decalcified freeze-dried bone allografts have osteogenic potential in periodontal bone defects.<sup>19</sup> Furthermore, the mean bone fill of the defect in one-wall (61%), two-wall (62%), wide three-wall (73%) and all defects combined (65%) is comparable to that reported by Froum et al.,<sup>24</sup> who used autogenous osseous coagulum-bone blend as the graft material with a similar experimental design. It is also interesting to note that approximately the same mean bone fill of the defect was obtained in one-wall and two-wall defects. However, the greatest percentage was obtained in wide three-wall defects. This is in agreement with the findings of Hiatt and Schallhorn<sup>25</sup> that the degree of osseous regeneration is directly proportional to the number of bony walls lining the defect.

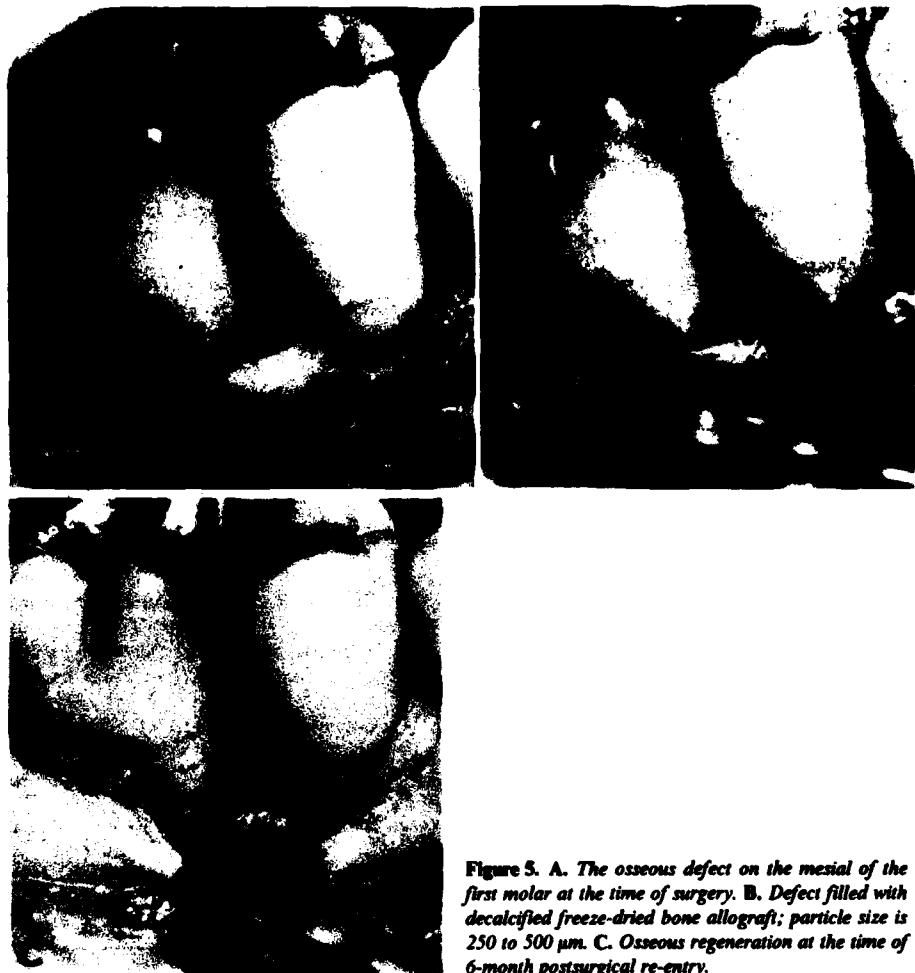


Figure 5. A. The osseous defect on the mesial of the first molar at the time of surgery. B. Defect filled with decalcified freeze-dried bone allograft; particle size is 250 to 500  $\mu\text{m}$ . C. Osseous regeneration at the time of 6-month postsurgical re-entry.

Table 2  
*Soft-Tissue Attachment Levels in Intraseptal Defects Treated With Decalcified Freeze-Dried Bone Allografts\**

Type of defects	No. of defects	Presurgical depth (mm)		Postsurgical depth (mm)†		Attachment gain (mm)	
		Mean	Range	Mean	Range	Mean	Range
One-wall	5	10.5	7.0-15.0	8.0	5.0-11.0	2.5	1.0-4.0
Two-wall	14	10.3	6.5-17.0	8.9	5.0-13.0	1.4	-1.0-7.0
Three-wall	8	9.8	6.0-14.0	7.9	5.0-11.0	1.9	-1.0-4.0
Total	27	10.2	6.0-17.0†	8.3	5.0-13.0‡	1.9	-1.0-7.0

\* Eleven subjects.

† Base of stent to base of pocket.

‡ Combined mean and range for the three types of defects.

Three of the 27 defects demonstrated crestal apposition of new bone after they had been grafted with decalcified freeze-dried bone allografts. Admittedly, the crestal apposition occurred in only a limited number of cases. Still, the potential for obtaining new bone coronal to the alveolar crest has been established.

Of importance also is the increase in clinical soft-tissue

attachment level that followed successful osseous reconstruction. It is tempting to speculate that this increase in attachment represented a new attachment composed of new bone, cementum and periodontal ligament fibers. Unfortunately, without histological data it is impossible to verify whether this type of attachment actually occurred.

A mean osseous regeneration of 65% suggests that decalcified freeze-dried bone allograft has some potential as a graft material in the treatment of periodontal osseous defects. Further evaluation of this material is indicated in an investigation that would include a greater number of grafted defects and ungrafted sites for comparison.

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